

1. Process for the reverse transcription and/or amplification of a product of a reverse transcription of a pool of nucleic acids of a type (A) from a biological sample or an enzymatic reaction, characterised by the selective suppression of the reverse transcription of at least one unwanted nucleic acid of type (A) and/or the selective suppression of the amplification of a product of a reverse transcription of at least one unwanted nucleic acid of type (A).  
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2. Process according to claim 1, characterised in that the nucleic acid of type (A) is mRNA.  
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3. Process according to claim 1, characterised in that the unwanted nucleic acid of type (A) is an mRNA which has a proportion of 20% or more of the total mRNA.  
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4. Process according to one of claims 2 to 3, comprising the following steps  
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  - a) carrying out a reverse transcription reaction of an RNA from a biological sample or a enzymatic reaction in the presence of at least one oligo-dT primer,  
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  - b) optionally after step a) carrying out a cDNA second strand synthesis,  
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  - c) optionally after step b) purifying the ds-cDNA while simultaneously depleting all the single-stranded nucleic acids from the reaction product of step b),  
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  - d) optionally after step a) and/or b) and/or c) carrying out amplification of the cDNA.  
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5. Process according to claim 4, characterised in that steps a) and/or d) are carried out in the presence of at least one molecular species for selectively suppressing the reverse transcription of at least one unwanted mRNA, while the molecular species prevents the reverse transcription of the unwanted mRNA, and/or for selectively suppressing the amplification of a product of the reverse transcription of at least one unwanted mRNA, the molecular species preventing the amplification of the single-stranded or double-stranded cDNA prepared from the unwanted mRNA.  
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6. Process according to one of claims 1 to 5, characterised in that in the reverse transcription reaction a reverse transcriptase with an intrinsic RNase H activity is used.
7. Process according to one of claims 1 to 6, characterised in that the biological sample is whole blood, muscle tissue or neuronal tissue, or it is a sample contaminated with whole blood, muscle tissue or neuronal tissue.
8. Process according to claim 7, characterised in that the biological sample is whole blood, and that the whole blood is taken up and/or stored in a stabilising reagent.
10. 9. Process according to claim 8, characterised in that the stabilising reagent is contained in a blood sample vial and the blood is transferred into the stabilising reagent immediately after being taken.
15. 10. Process according to claim 8 or 9, characterised in that the stabilising reagent contains a tetra-alkyl-ammonium salt in the presence of an organic acid.
11. Process according to claim 8 or 9, characterised in that the stabilising reagent contains at least one guanidine compound, a buffer substance, a reducing agent and a detergent.
20. 12. Process according to one of claims 1 to 11, characterised in that the biological sample is whole blood, and that the unwanted nucleic acid of type (A) is globin-mRNA.
25. 13. Process according to claim 4, characterised in that in order to purify a ds-cDNA in step c) first of all the nucleic acids obtained from step b) and/or those obtained from the optional step d) are bound in their entirety to a silica matrix and then the silica matrix is washed with a guanidine-containing washing buffer to deplete the single-stranded nucleic acids.
30. 14. Process according to claim 13, characterised in that the silica matrix used consists of one or more silica membrane(s) or silica particles, particularly magnetic silica particles.

15. Process according to claim 13, characterised in that the guanidine-containing washing buffer contains guanidine isothiocyanate and/or guanidine thiocyanate in a concentration of 1 M to 7 M, preferably 2.5 M to 6 M and particularly preferably 3 M to 5.7 M.
- 5     16. Process according to claim 13, characterised in that the guanidine-containing washing buffer contains guanidine hydrochloride in a concentration of 4 M to 9 M, preferably 5 M to 8 M.
- 10    17. Process according to claim 5, characterised in that the molecular species is a DNA oligonucleotide and/or RNA oligonucleotide complementary to the mRNA or to one of the cDNA strands, or a corresponding oligonucleotide from DNA and/or RNA derivatives, or a corresponding DNA and/or RNA oligonucleotide containing modified or artificial nucleotides, quenchers or fluorophores.
- 15    18. Process according to claim 17, characterised in that the molecular species has a length of 10 to 60 nucleotides, preferably 12 to 30 nucleotides.
- 20    19. Process according to claim 5, characterised in that the molecular species is a nucleic acid analogue complementary to the mRNA or to one of the cDNA strands.
- 25    20. Process according to claim 19, characterised in that the nucleic acid analogue is PNA, LNA or GripNA.
- 30    21. Process according to claim 20, characterised in that the PNA has a length of 12 to 20 nucleotide analogues, preferably 13 to 16 nucleotide analogues.
22. Process according to claim 20, characterised in that the LNA comprises at least one nucleotide which is a 'locked nucleotide', and that the LNA has a length of 14 to 30 nucleotides, preferably 15 to 22 nucleotides.
23. Process according to claim 20, characterised in that the GripNA has a length of 12 to 30 nucleotide analogues, preferably 14 to 20 nucleotide analogues.

24. Process according to one of claims 17 to 23, characterised in that the molecular species binds in the 3' region of the mRNA or one of the cDNA strands.

5 25. Process according to one of claims 5 and 17 to 24, characterised in that a number of molecular species are used which are complementary to different regions of one or more specific mRNA(s) or at least one strand of one or more specific cDNA(s).

10 26. Process according to one of claims 5 and 17 to 24, characterised in that at least one molecular species is used which is complementary to a homologous region of different mRNAs or cDNAs.

15 27. Process according to one of claims 5 and 17 to 26, characterised in that the molecular species has at its 3' end a modification which prevents elongation from being initialised at the 3' end of the molecular species.

28. Process according to claim 5, characterised in that the molecular species is a ribozyme.

20 29. Process according to claim 28, characterised in that the molecular species is a hammerhead ribozyme or a hairpin ribozyme.

30. Process according to claim 28 or 29, characterised in that the ribozyme consists of RNA or an RNA derivative or embodies fusion products of such ribozymes.

25 31. Process according to one of claims 28 to 30, characterised in that the sequence of the ribozymes complementary to the unwanted mRNA or cDNA has a length of 12 to 30 nucleotides, preferably 15 to 25 nucleotides.

32. Process according to claim 5, characterised in that the molecular species is a DNAzyme.

30 33. Process according to one of claims 5, 12 and 17, characterised in that the molecular species is a DNA oligonucleotide and the globin-mRNA embodies an alpha 1 globin-

mRNA and/or an alpha 2 globin-mRNA, the DNA oligonucleotide comprising a sequence selected from among

- 5 a) 5' CTC CAG CTT AAC GGT - phosphate group - 3'
- b) 5' TAA CGG TAT TTG GAG - phosphate group - 3'
- c) 5' TAA CGG TAT TTG GAG GTC AGC ACG GTG CTC - phosphate group - 3'.

34. Process according to one of claims 5, 12 and 17, characterised in that the molecular  
10 species is a DNA-oligonucleotide and the globin-mRNA embodies a beta globin-mRNA,  
the DNA-oligonucleotide comprising a sequence selected from among

- a) 5' GTA GTT GGA CTT AGG - phosphate group - 3'
- b) 5' ATC CAG ATG CTC AAG - phosphate group - 3'
- 15 c) 5' GTA GTT GGA CTT AGG GAA CAA AGG AAC CTT - phosphate group - 3'.

35. Process according to one of claims 5, 12 and 20, characterised in that the molecular  
species is a PNA and the globin-mRNA embodies an alpha 1 globin-mRNA and/or an  
20 alpha 2 globin-mRNA, the PNA comprising a sequence selected from among

- a) N- CTC CAG CTT AAC GGT -C\*
- b) N- TAA CGG TAT TTG GAG -C\*
- c) N- GTC ACC AGC AGG CA -C\*
- 25 d) N- GTG AAC TCG GCG -C\*
- e) N- TGG CAA TTC GAC CTC -C\*
- f) N- GAG GTT TAT GGC AAT -C\*
- g) N- ACG GAC GAC CAC TG -C\*
- h) N- GCG GCT CAA GTG -C\*.

30 36. Process according to one of claims 5, 12 and 20, characterised in that the molecular  
species is a PNA and the globin-mRNA embodies a beta globin-mRNA, the PNA  
comprising a sequence selected from among

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- a) N- GTA GTT GGA CTT AGG -C\*
- b) N- ATC CAG ATG CTC AAG -C\*
- c) N- CCC CAG TTT AGT AGT -C\*
- d) N- CAG TTT AGT AGT TGG -C\*
- e) N- GCC CTT CAT AAT ATC -C\*
- f) N- GGA TTC AGG TTG ATG -C\*
- g) N- GAA CTC GAT GAC CTA -C\*
- h) N- TGA TGA TTT GAC CCC -C\*
- i) N- GGT TGA TGA TTT GAC -C\*
- 10 j) N- CTA TAA TAC TTC CCG -C\*.

37. Process according to one of claims 5, 12 and 20, characterised in that the molecular species is an LNA comprising at least one nucleotide which is a 'locked nucleotide' and the globin-mRNA is an alpha 1-globin-mRNA and/or an alpha 2-globin-mRNA, the LNA comprising a sequence selected from among

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- a) 5' CTC CAG CTT AAC GGT - octanediol - 3'
- b) 5' TAA CGG TAT TTG GAG - octanediol -3'
- c) 5' GTC ACC AGC AGG CA - octanediol -3'
- 20 d) 5' GTG AAC TCG GCG - octanediol -3'.

38. Process according to one of claims 5, 12 and 20, characterised in that the molecular species is an LNA, comprising at least one nucleotide which is a 'locked nucleotide', and the globin-mRNA embodies a beta globin-mRNA, the LNA comprising a sequence selected from among

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- a) 5' GTA GTT GGA CTT AGG - octanediol -3'
- b) 5' ATC CAG ATG CTC AAG - octanediol -3'
- c) 5' CCC CAG TTT AGT AGT - octanediol -3'
- 30 d) 5' CAG TTT AGT AGT TGG - octanediol -3'
- e) 5' GCC CTT CAT AAT ATC - octanediol -3'.

39. Process according to one of claims 1, 4 and 5, characterised in that the amplification comprises *in vitro* transcription.

40. Process according to claim 39, characterised in that the *in vitro* transcription is followed by a DNase digestion as well as purification of the cRNA.

41. Use of cRNA resulting from a process according to claim 39 or 40 in a gene expression analysis.

10 42. Use of cDNA resulting from a process according to one of claims 1 to 38 in a gene expression analysis.

43. Use of a washing buffer containing at least one guanidine compound in a total concentration of 1 M to 9 M, wherein the guanidine compound is guanidine isothiocyanate in a concentration of 1 M to 7 M, preferably 2.5 M to 6 M and particularly preferably 3 M to 5.7 M, and/or the guanidine compound is guanidine thiocyanate in a concentration of 1 M to 7 M, preferably 2.5 M to 6 M and particularly preferably 3 M to 5.7 M and/or the guanidine compound is guanidine hydrochloride in a concentration of 4 M to 9 M, preferably 5 M to 8 M, in a process for separating single-stranded nucleic acids from double-stranded nucleic acids, preferably in a process for eluting single-stranded nucleic acids from a silica matrix and particularly preferably in a process according to one of claims 1 to 16.

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44. Use according to claim 43, wherein the washing buffer further contains one or more buffer substance(s) in a total concentration of 0 mM to 40 mM and/or one or more additive(s) in a total concentration of 0 mM to 100 mM and/or one or more detergent(s) in a total concentration of 0 %(v/v) to 20 %(v/v), the washing buffer having a pH of 5 to 9.

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30 45. Use according to claim 44, characterised in that the total concentration of the buffer substances is 20 mM to 40 mM.

46. Use according to claim 44 or 45, characterised in that the buffer substance is Tris, Tris-HCl, MOPS, MES, CHES, HEPES, PIPES and/or sodium citrate.

47. Use according to claim 44, characterised in that the additive is a chelating agent.

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48. Use according to claim 47, characterised in that the chelating agent is EDTA and/or EGTA.

49. Use according to claim 44, characterised in that the detergent is Tween 20, Triton X 100,  
10 Sarcosyl and/or NP 40.

50. Use according to claim 44, characterised in that the pH value of the washing buffer is in  
the range from 6 to 8.

15 51. Use according to one of claims 43 to 46, characterised in that the guanidine-compound is  
guanidine isothiocyanate and/or guanidine thiocyanate and the buffer substance is sodium  
citrate, while the washing buffer has a pH in the range from 6 to 8.

20 52. Use according to claim 51, characterised in that the washing buffer contains guanidine  
isothiocyanate and/or guanidine thiocyanate in a total concentration of 3.2 M to 3.8 M as  
well as sodium citrate in a concentration of 20 mM to 30 mM and has a pH of 6.8 to 7.7.

25 53. Use according to claim 52, characterised in that the washing buffer contains guanidine  
isothiocyanate and/or guanidine thiocyanate in a total concentration of 3.5 M as well as  
sodium citrate in a concentration of 25 mM and has a pH of 7.0.